

PATENT ABSTRACTS OF JAPAN

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(71)Applicant : SHOWA SHELL SEKIYU KK

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(72)Inventor : MATSUMOTO TAKAO
OTA TORU
SADAMUNE YASUNORI

(54) BIODEGRADATION TESTING OF OIL MATTER

(57)Abstract:

PURPOSE: To provide a simple method of biodegradation testing for simply and safely evaluating the biodegradation of an oil matter such as lubricating oil or grease in a short period of time, and for testing a number of materials to be tested at the same time.

CONSTITUTION: To test the biodegradation of oil matter, an oil matter to be tested and a microorganism source are added in a culture solution including an organic nutrition source, which is cultured. After an organic solvent is added in the culture solution, and they are sufficiently mixed together, the water phase and the organic solvent layer are separated from one another, to determine the amount of biodegradation of the oil matter which is not decomposed included in the organic solvent layer. The amount of a degradation composition in the oil material to be tested, is thus calculated.

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CLAIMS

[Claim(s)]

[Claim 1] The biodegradability examining method of the oil characterized by separating the aqueous phase and an organic solvent layer, calculating the amount of the subject oil of not decomposing [which is included in the organic solvent layer], and computing the amount of the biodegradation component in subject oil from this after cultivating by adding subject oil and the source of a microorganism, adding an organic solvent subsequently to culture medium into culture medium including the source of heterotrophism and mixing enough.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the simple biodegradability examining method for oily nature matter, such as various lubricating oils which are oily nature and are characterized by being mixture, or grease.

[0002]

[Description of the Prior Art] In recent years, also in a petroleum product, the needs of an environment-friendly product are increasing from a global rise of global environment problems. For example, generally 2 stroke engine is used, a non-burned lubricating oil is discharged with exhaust gas, and, as for the outboard motor of a motorboat, **** contamination of the hydrosphere of the sea, a lake, etc. has been a problem. Moreover, in forestry, the **** contamination to the forest soil system of the lubricating oil discharged from the chain saw currently used for felling of a tree poses a problem. In an environment-friendly nature, it may decompose promptly, that is, commercialization of a lubricating oil or grease with biodegradability is wished also that of such a background. By the way, some kinds, such as a biochemical-oxygen-demand (BOD) measuring method, a generating carbon-dioxide measuring method, a dissolved-organic-carbon measuring method, and specific analysis methods (a gas chromatography, high performance chromatography, etc.) of an object chemical, are developed, and the biodegradability test method using the microorganism of a chemical is adopted as an OECD chemistry article test guideline. Generally, from the physical properties of an object chemical etc., the optimal biodegradability test method is chosen from the above, and is performed. However, when the above-mentioned test method estimates the biodegradability of a lubricating oil, grease, etc., they are water-insoluble nature, and since they are mixture, they are difficult in many cases. Moreover, special equipment, an instrument, and the skilled skill are required, and, as for these, the need etc. usually has some faults for one month in the trial further. On the other hand, current and the biodegradability test method of a lubricating oil are CEC generally specified to CEC (Europe specification committee). - T-L-33 82 law (henceforth the CEC method) is used. Using the primary treatment water or the secondary effluent of a city sewage final sewage disposal plant as a CEC method sowing bacillus, culture is performed for about one month, the organic solvent of the halogen system which does not include C-H coupling, such as a carbon tetrachloride, extracts culture medium at the time of culture termination, by measuring the absorbance of 2930cm⁻¹ for an extract using an infrared spectrophotometer, the quantum of the amount of residual oil is carried out, and biodegradability is searched for. Thus, it is specified that the halogen system organic solvent with which use is being restricted detrimentally and globally to the body is used for the CEC method so much. Moreover, the duration of test requires about one month like the various biodegradability test methods specified to the above-mentioned OECD chemistry article test guideline, the skilled skill is required and dispersion in a measurement result cannot be disregarded, either. Furthermore, examining many examined substances at once has the fault of difficulty etc.

[0003]

[Objects of the Invention] The purpose of this invention can evaluate the biodegradability of oil,

such as a lubricating oil and grease, safely simply again for a short period of time, and is to offer the simple biodegradability test method which can examine many examined substances at once further.

[0004]

[Elements of the Invention] As a carbon source (energy source of a microorganism), for a microorganism, only the subject chemical or the lubricating oil is added, and it decomposes, and since the biodegradability test method and the CEC method which are specified to the OECD chemistry article test guideline are a method of examining under ***** conditions, a trial takes them for a long period of time. They present the shape of an oil, and quanta, such as a lubricating oil which is an examined substance, or grease, are mixture in many cases, and specific analytical method or dissolved-organic-carbon methods, such as a gas chromatography and high performance chromatography, etc. are unsuitable. Then, by using the growth culture system which added the nutriment for a microorganism to the biodegradability trial culture medium, this invention person etc. tried compaction of the biodegradability duration of test, and elaborated the extract approach. That is, after cultivating by adding subject oil and the source of a microorganism, adding an organic solvent subsequently to culture medium into culture medium including the source of heterotrophism and mixing enough, this invention separates the aqueous phase and an organic solvent layer, calculates the amount of the non-decomposing oil contained in the organic solvent layer, and relates to the biodegradability examining method of the oil characterized by computing the amount of the biodegradation component in subject oil from this. That is, this invention adds organic solvents, such as a hexane of optimum dose, for the culture medium containing the oil at the time of test termination, separates the aqueous phase and an organic solvent layer, and performs them by measuring the weight of the amount of residual oil after removing an organic solvent. It can ask for the rate of biodegradation by / (decomposition oil quantity) (initiation addition).

[0005] This invention is explained further below at a detail.

1. the source of a microorganism used as seeding seeding -- either the surface layer water of the primary treatment water (beginning setting tank passage liquid) of a city sewage disposal plant, the active sludge from a city sewage disposal plant aerator, soil suspension, a river, and a lake, or such mixture -- although -- it can use. The microorganism concentration of these seeding has 10⁶ CFU(s)/desirable more than ml. Moreover, it is desirable to use within 12 hours after seeding preparation.

[0006] 2. A peptone, a yeast extract, a meat extract, corn steep liquor, a malt extract, etc. can be used as a source of medium composition heterotrophism. The addition concentration to culture medium has 0.05 - 1.0% of the weight of the desirable range. As mineral salt, a phosphoric acid potassium, an ammonium sulfate, magnesium sulfate, an ammonium chloride, a calcium chloride, etc. are used. Addition concentration has 0.01 - 0.1% of the weight of the desirable range. Trace elements, such as copper, iron, zinc, manganese, cobalt, and molybdenum, can be added if needed.

[0007] 3. Add 100-200mg of subject oil directly to 500ml ***** or the Sakaguchi flask containing 50-200ml of culture medium prepared by biodegradability test-method 2., or subject oil concentration adds preferably n-hexane or the acetone solution prepared [ml] in 100-200mg /to culture medium by the capacity of 1ml or less. Next, seeding prepared by 1. is added in 1-20ml. A cotton plug or a SHIRIKO plug is made a flask, and, in the case of an Erlenmeyer flask, in the case of a rotary-shaking-culture machine and a Sakaguchi flask, shaking culture is performed using a reciprocal-shaking-culture machine. A culture condition is performed in the range with a temperature of 20-37 degrees C by the dark condition. The flask which does not add seeding to oil additive-free a flask and subject oil as contrast cultivates similarly. Since the flask which does not add seeding is what considers physicochemical change in which microbial degradation does not participate, it needs sterile actuation. The duration of test is performed in the range for three - seven days. Moreover, it is desirable to perform each trial flask by two or more reams if needed.

[0008] 4. Add several drops of concentrated hydrochloric acid in the flask containing the culture medium which ended culture by oil assay 3., and make pH of culture medium into acidity. next,

organic solvents, such as a hexane, a pentane, benzene, and ethyl acetate, -- preferably, it is easy to add and mixes with 20 ml of n-hexanes. Separation of the aqueous phase and an organic solvent phase has desirable centrifugal separation, although centrifugal separation, a separating funnel, a hydrophobic filter paper, etc. may use any. The organic solvent whole quantity is carefully moved to the evaporating dish which has carried out weighing capacity beforehand so that the aqueous phase may not mix, and an organic solvent is made to transpire under about 80-degree C conditions on a hot plate. It cools radiationally after evapotranspiration and within a desiccator for about 30 minutes, and weighing capacity of the weight of the evaporating dish containing oil is carried out.

[0009] 5. Ask for calculation of the rate of calculation biodegradation of the rate of biodegradation by the following formulas (I).

[Equation 1]

$$\text{生分解率 (\%)} = \left(1 - \frac{S_t - B_t}{S_o - B_o} \right) \times 100 \quad \dots\dots (I)$$

Here, it is S_t : residual oil weight (mg).

S_o : The 0th hour oil weight (mg)

B_t : Organic solvent extract weight in an oil additive-free system (mg)

B_o : Organic solvent extract weight in the 0th hour oil additive-free system (mg)

[0010]

[Example] Below, the example of this invention is explained concretely. In addition, these examples are for illustrating this invention, and do not limit the range of this invention. The straight mineral oil and synthetic oil (Table 1) which are used as a base oil of various lubricating oils and grease as example 1 sample-offering oil were used.

[Table 1]

表 1

油 種 系 統	供試ベースオイル
パラフィン系鉱油	HVI 60 HVI 650
ナフテン系鉱油	MVI N40 MVI N170
ポリオールエステル系合成油	Priolube 1426 Priolube 3999 Di-iso-tridecyl adipate KSL 224
二塩基酸エステル系合成油	DOA
芳香族エステル系合成油	Reolube OTM
ポリ α オレフィン系合成油	LUCANT HC10

The source of a microorganism used as seeding used the primary treatment water of the Sagami river terminal sewage disposal plant. The cell mass concentration of seeding was 3.9×10^7 CFU/ml. The medium composition made addition concentration 0.1%, using a peptone as a source

of heterotrophism. Other additional mineral salt and a trace element were shown in Table 2.
[Table 2]

表 2

培養液組成	添加濃度 (mg/リットル)
KH_2PO_4	8.50
K_2HPO_4	21.75
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	33.40
NH_4Cl	15.00
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	22.50
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	36.40
H_3BO_3	0.75
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	3.00
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.10
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.50
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.05
$\text{CoSO}_4 \cdot 2\text{H}_2\text{O}$	0.10
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.05
Peptone	1.0g/リットル

The Erlenmeyer flask containing 100ml of culture medium prepared above performed the biodegradability trial. Sample offering oil dissolved in n-hexane so that it might become [ml] in 200mg /, and it added 1ml of this hexane solution in each flask. Seeding added 10ml in each flask, and started the biodegradability trial. The rotary-shaking-culture machine performed the culture condition on rotational frequency 220rpm, a dark place, and 25-degree C conditions. The biodegradability trial was performed for four days and the culture medium whole quantity of each flask was used for the following oil quantum.

[Table 3]

表 3

供試物質	本法での生分解率 (%)	CEC法での生分解率 (%)
HVI 60	30.7	41.4
HVI 650	24.8	14.8
MVI N40	24.5	0.0
MVI N170	2.8	18.1
Priolube 1426	98.2	100.0
Priolube 3999	85.3	99.0
Di-iso-tridecyl adipate	59.5	89.9
KSL 224	95.7	100.0
DOA	98.2	100.0
Reolube OTM	12.2	0.0
LUCANT HC10	4.2	5.7

The quantum of oil was performed by the following approaches. Several drops of concentrated

hydrochloric acid was added to the Erlenmeyer flask containing the culture medium which ended culture, and pH of culture medium was made into acidity. Next, n-hexane was added in the flask, it mixed well, and the whole quantity was moved to the centrifugation tube. Furthermore, n-hexane 20ml was added in the flask, the inside of a flask was washed, and the washings was united. At long-intervals alignment separation was carried out for this mixture for 10,000rpm and 10 minutes, and two phases were made to separate. It moved so that water might not mix n-hexane phase in the evaporating dish which has carried out weighing capacity beforehand with Pasteur pipette, and n-hexane was made to transpire under about 80-degree C conditions on a hot plate. It cooled radiationally for 30 minutes after evapotranspiration and within the desiccator, and the weight of the evaporating dish containing oil was measured. It asked for calculation of the rate of biodegradation by the formula (I). Next, the rate of day [of culture / 4th] biodegradation is shown in Table 3. In addition, the rate of biodegradation in the 21st day in the CEC method was collectively specified in Table 3. In comparison with the rate of biodegradation obtained by the CEC method from Table 3, although a gap of some value is accepted, it can be said that the test result reflecting the inclination of biodegradability was obtained. That is, the rate of biodegradation with the low difficulty biodegradability matter was obtained at the rate of biodegradation with the expensive ready biodegradability matter. Moreover, with coefficient of variation, the repeat analysis precision in this method becomes 10% or less with coefficient of variation, repeatability becomes 20% or less, and a high precision is acquired as a precision of a biodegradability trial. Thus, by using this invention, as for the difficult lubricating oil, grease, etc., it is oily to **** biodegradability, it can carry out very much for a short period of time, and the thing of four days which do not use a halogen system organic solvent further and which is performed safely and simply can perform the place which required the biodegradability of mixture for one month conventionally.

[0011]

[Effect] This invention was the thing of precision with the data simply, quite expensive for a short period of time, while being able to **** safely obtained by that cause about the biodegradability of oil, such as a lubricating oil and grease.

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TECHNICAL FIELD

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PRIOR ART

[Description of the Prior Art] In recent years, also in a petroleum product, the needs of an environment-friendly product are increasing from a global rise of global environment problems. For example, generally 2 stroke engine is used, a non-burned lubricating oil is discharged with exhaust gas, and, as for the outboard motor of a motorboat, **** contamination of the hydrosphere of the sea, a lake, etc. has been a problem. Moreover, in forestry, the **** contamination to the forest soil system of the lubricating oil discharged from the chain saw currently used for felling of a tree poses a problem. In an environment-friendly nature, it may decompose promptly, that is, commercialization of a lubricating oil or grease with biodegradability is wished also that of such a background. By the way, some kinds, such as a biochemical-oxygen-demand (BOD) measuring method, a generating carbon-dioxide measuring method, a dissolved-organic-carbon measuring method, and specific analysis methods (a gas chromatography, high performance chromatography, etc.) of an object chemical, are developed, and the biodegradability test method using the microorganism of a chemical is adopted as an OECD chemistry article test guideline. Generally, from the physical properties of an object chemical etc., the optimal biodegradability test method is chosen from the above, and is performed. However, when the above-mentioned test method estimates the biodegradability of a lubricating oil, grease, etc., they are water-insoluble nature, and since they are mixture, they are difficult in many cases. Moreover, special equipment, an instrument, and the skilled skill are required, and, as for these, the need etc. usually has some faults for one month in the trial further. On the other hand, current and the biodegradability test method of a lubricating oil are CEC generally specified to CEC (Europe specification committee). - T-L-33 82 law (henceforth the CEC method) is used. Using the primary treatment water or the secondary effluent of a city sewage final sewage disposal plant as a CEC method sowing bacillus, culture is performed for about one month, the organic solvent of the halogen system which does not include C-H coupling, such as a carbon tetrachloride, extracts culture medium at the time of culture termination, by measuring the absorbance of 2930cm⁻¹ for an extract using an infrared spectrophotometer, the quantum of the amount of residual oil is carried out, and biodegradability is searched for. Thus, it is specified that the halogen system organic solvent with which use is being restricted detrimentally and globally to the body is used for the CEC method so much. Moreover, the duration of test requires about one month like the various biodegradability test methods specified to the above-mentioned OECD chemistry article test guideline, the skilled skill is required and dispersion in a measurement result cannot be disregarded, either. Furthermore, examining many examined substances at once has the fault of difficulty etc.

[0003]

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[0004]

[Elements of the Invention] As a carbon source (energy source of a microorganism), for a microorganism, only the subject chemical or the lubricating oil is added, and it decomposes, and

since the biodegradability test method and the CEC method which are specified to the OECD chemistry article test guideline are a method of examining under ***** conditions, a trial takes them for a long period of time. They present the shape of an oil, and quanta, such as a lubricating oil which is an examined substance, or grease, are mixture in many cases, and specific analytical method or dissolved-organic-carbon methods, such as a gas chromatography and high performance chromatography, etc. are unsuitable. Then, by using the growth culture system which added the nutriment for a microorganism to the biodegradability trial culture medium, this invention person etc. tried compaction of the biodegradability duration of test, and elaborated the extract approach. That is, after cultivating by adding subject oil and the source of a microorganism, adding an organic solvent subsequently to culture medium into culture medium including the source of heterotrophism and mixing enough, this invention separates the aqueous phase and an organic solvent layer, calculates the amount of the non-decomposing oil contained in the organic solvent layer, and relates to the biodegradability examining method of the oil characterized by computing the amount of the biodegradation component in subject oil from this. That is, this invention adds organic solvents, such as a hexane of optimum dose, for the culture medium containing the oil at the time of test termination, separates the aqueous phase and an organic solvent layer, and performs them by measuring the weight of the amount of residual oil after removing an organic solvent. It can ask for the rate of biodegradation by / (decomposition oil quantity) (initiation addition).

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[0010]

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Di-iso-tridecyl adipate	59.5	89.9
KSL 224	95.7	100.0
DOA	98.2	100.0
Reolube OTM	12.2	0.0
LUCANT HC10	4.2	5.7

The quantum of oil was performed by the following approaches. Several drops of concentrated hydrochloric acid was added to the Erlenmeyer flask containing the culture medium which ended culture, and pH of culture medium was made into acidity. Next, n-hexane 20ml was added in the

flask, it mixed well, and the whole quantity was moved to the centrifugation tube. Furthermore, n-hexane 20ml was added in the flask, the inside of a flask was washed, and the washings was united. At long-intervals alignment separation was carried out for this mixture for 10,000rpm and 10 minutes, and two phases were made to separate. It moved so that water might not mix n-hexane phase in the evaporating dish which has carried out weighing capacity beforehand with Pasteur pipette, and n-hexane was made to transpire under about 80-degree C conditions on a hot plate. It cooled radiationally for 30 minutes after evapotranspiration and within the desiccator, and the weight of the evaporating dish containing oil was measured. It asked for calculation of the rate of biodegradation by the formula (1). Next, the rate of day [of culture / 4th] biodegradation is shown in Table 3. In addition, the rate of biodegradation in the 21st day in the CEC method was collectively specified in Table 3. In comparison with the rate of biodegradation obtained by the CEC method from Table 3, although a gap of some value is accepted, it can be said that the test result reflecting the inclination of biodegradability was obtained. That is, the rate of biodegradation with the low difficulty biodegradability matter was obtained at the rate of biodegradation with the expensive ready biodegradability matter. Moreover, with coefficient of variation, the repeat analysis precision in this method becomes 10% or less with coefficient of variation, repeatability becomes 20% or less, and a high precision is acquired as a precision of a biodegradability trial. Thus, by using this invention, as for the difficult lubricating oil, grease, etc., it is oily to **** biodegradability, it can carry out very much for a short period of time, and the thing of four days which do not use a halogen system organic solvent further and which is performed safely and simply can perform the place which required the biodegradability of mixture for one month conventionally.

[Translation done.]

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